In light of the amendments and remarks presented below, Applicants request withdrawal of the rejections and favorable reconsider of the application.

AMENDMENT

In the Specification:

Please amend the substitute specification as follows:

Please replace the paragraph beginning at page 21, line 4, with the following rewritten paragraph:

E1

--One class of molecules that could be useful in this type of application are receptors. For example, a specific receptor could be displayed on the surface of the phage such that it would bind its ligand. The receptor could then be modified by, for example, in vitro mutagenesis and variants having higher binding affinity for the ligand selected. The selection may be carried out according to one or more of the formats described below with reference to figure 2A and figure 2B (which refers particularly to pAbs) in which the pAb antibody is replaced with a phage receptor and the antigen with a ligand l.--

Please replace the paragraph beginning at page 42, line 6, with the following rewritten paragraph:

E2

--The rgdp may be a bacteriophage, the host a bacterium, and said component of the rgdp a capsid protein for the bacterophage. The phage may be a filamentous phage. The phage may be selected from the class I phages fd, M13, f1, Ifl, lke, ZJ/Z, Ff and the class II phages Xf, Pf1 and Pf3. The phage may be fd or a derivative of fd. The derivative may be tetracycline resistant. The said sbp member or polypeptide chain thereof may be expressed as a fusion with the gene III capsid protein of phage fd or its counterpart in another filamentous phage. The sbp member or polypeptide chain thereof may be inserted in the N-terminal region of the mature capsid protein downstream of a secretory leader peptide. The sequence may be inserted after amino acid +1 of the mature protein. The site for insertion may be flanked by short sequences corresponding to sequences which occur at each end of the nucleic acid to be inserted. For example, where the protein domain is an immunoglobulin domain, the insertion site in the phage may be flanked by nucleotide sequences

which code for the first five amino acids and the last five amino acids of the Ig domain. Such flanking nucleotide sequences are shown in figure 4B line B and line C, wherein the site-flanking nucleotide sequences encode amino acid sequences QVQLQ and VTVSS which occur at either end of the VH domain, or QVQLQ and LEIKR which occur at either end of the Fv (combined VH + VL) domain. Each of these sequences flanking the insertion site may include a suitable cleavage site, as shown in figure 4A and figure 4B.--

Please replace the paragraph beginning at page 43, line 8, with the following rewritten paragraph:

--Alternatively, the flanking nucleotide sequences shown in figure 4B line B and line C as described above, may be used to flank the insertion site for any nucleic acid to be inserted, whether or not that nucleic acid codes an immunoglobulin.--

Please replace the paragraph beginning at page 58, line 4, with the following rewritten paragraph:

--The applicants have also devised a series of novel selection techniques that are practicable only because of the unique properties of rgdps. The general outline of some screening procedures is illustrated in figure 2A and figure 2B using pAbs as an example type of rgdp.--

Please replace the paragraph beginning at page 58, line 10, with the following rewritten paragraph:

--The population/library of pAbs to be screened could be generated from immunised or other animals; or be created in vitro by mutagenising pre-existing phage antibodies (using techniques well-known in the art such as oligonucleotide directed mutagenesis (Sambrook, J., et al., 1989 Molecular Cloning a Laboratory Manual, Cold Spring Harbor Laboratory Press). This population can be screened in one or more of the formats described below with reference to figure 2A and figure 2B, to derive those individual pAbs whose antigen binding properties are different from sample c.--

Please replace the paragraph beginning at page 58, line 23, with the following rewritten paragraph:

--Figure 2A shows antigen (ag) bound to a solid surface (s) the solid surface (s) may be provided by a petri dish, chromatography beads, magnetic beads and the like. The population/library of pAbs is then passed over the ag, and those individuals p that bind are retained after washing, and optionally detected with detection system d. A detection system based upon anti-fd antisera is illustrated in more detail below in example 4. If samples of bound population p are removed under increasingly stringent conditions, the binding affinity represented in each sample will increase. Conditions of increased stringency can be obtained, for example, by increasing the time of soaking or changing the pH of the soak solution, etc.--

Please replace the paragraph beginning at page 59, line 10, with the following rewritten paragraph:

--Referring to figure 2B antigen (ag) can be bound to a solid support s and bound to saturation by the original binding molecule c. If a population of mutant pAb (or a set of unrelated pAbs) is offered to the complex, only those that have higher affinity for antigen ag than c will bind. In most examples, only a minority of population c will be displaced by individuals from population p. If c is a traditional antibody molecule, all bound material can be recovered and bound p recovered by infecting suitable bacteria and/or by use of standard techniques such as PCR.--

Please replace the paragraph beginning at page 65, line 23, with the following rewritten paragraph:

--Figure 2A and Figure 2B show schematically selection techniques which utilise the unique properties of pAbs; figure 2A shows a binding/elution system; and figure 2B shows a competition system (p=pAb; ag=antigen to which binding by pAb is required; c=competitor population e.g. antibody, pAb, ligand; s=substrate (e.g. plastic beads etc); d=detection system.--

Please replace the paragraph beginning at page 66, line 5, with the following rewritten paragraph:

--Figure 4A and Figure 4B show the nucleotide sequences for the oligonucleotides and vectors. All sequences are drawn 5' to 3' and are numbered according to Beck et al., 1978, Nucl. Acid Res., 5: 4495-4503. Figure 4A shows the sequences of the oligonucleotides used for mutagenesis (oligo's 1 and 2) or sequencing (oligo 3). The sequences shown were synthesized on an Applied Biosystems, oligonucleotide synthesizer and are complementary to the single stranded form of fd-tet (they are in the anti-sense form with respect to gene III). Figure 4B shows the sequences of the various constructs around the gene III insertion site. These sequences are drawn in the sense orientation with respect to gene III; line A fd-tet (and fdT Bst) line B fdTPs/Bs and line C fdTPs/Xh. The key restriction enzyme sites are shown along with the immunoglobulin amino acids contributed by the vectors, (amino acid single letter code is used, see Harlow, E., and Lane, D., 1988 supra.).--

Please replace the paragraph beginning at page 66, line 24, with the following rewritten paragraph:

--Figure 5A and Figure 5B show the nucleotide and amino acid sequences for scFv in the vector scFvD1.3 myc. This gives the sequence of the anti-lysozyme single chain Fv and surrounding sequences in scFvD1.3 myc, showing the N-terminal pel B signal peptide sequence and the C-terminal myc tag sequence (Ward, E.S., et al., 1989, supra.). Also shown is the peptide sequence linking the VH and VL regions. The amino acid sequence is represented above the nucleotide sequence by the single letter code, see Harlow, E., and Lane D., 1988 supra.--

Please replace the paragraph beginning at page 67, line 29, with the following rewritten paragraph:

--Figure 10A through Figure 10C show the contiguous sequence of FabD1.3. Figure 10D shows a map of FabD1.3 in pUC19.--

Please replace the paragraph beginning at page 69, line 1, with the following rewritten paragraph:

E12 Sub 6/10

--Figure 16A shows the structure of gene III and the native BamHI site into which a scFv coding sequence was inserted in example 13 and figure 16B shows the natural peptide linker sites A and B for possible insertion of scFv coding sequences.--

Please replace the paragraph beginning at page 70, line 15, with the following rewritten paragraph:

E13

--Figure 24A through figure 24D show VH and VK gene sequences derived from the combinatorial library in example 21 and the hierarchical library in example 22.--

Please replace the paragraph beginning at page 70, line 18, with the following rewritten paragraph:

E14

--Figure 25 shows a matrix of ELISA signals for clones derived from random combinatorial library. Designation of the clones is as in figure 24A through figure 24D. The number of clones found with each combination is shown by the numerals.--

Please replace the paragraph beginning at page 76, line 8, with the following rewritten paragraph:

E15

--Figure 44A and Figure 44B show the DNA sequence of scFv B18 (anti-NP).--

Please replace the paragraph beginning at page 76, line 23, with the following rewritten paragraph:

E16

--Figure 48A, Figure 48B, and Figure 48C show a map of plasmid pJM1-FabD1.3 which is used for the expression of soluble human Fab fragments and as a template for the synthesis of linker DNA for Fab assembly. B. a schematic representation of sequences encoding a Fab construct. C. The sequence of DNA template for the synthesis of linker DNA for Fab assembly.--

Please replace the paragraph beginning at page 77, line 5, with the following rewritten paragraph:

E17 --Figure 50A and Figure 50B show an ELISA assay of phage antibodies using plates coated with turkey egg lysozyme. Two clones B1 and A4 are shown derived by mutagenesis and selection from pAbD1.3 (example 45). Concentration (x axis) refers to the concentration of phage for each sample relative to the concentration in culture supernatant. B1 has raised binding to turkey egg lysozyme compared to D1.3. A4 has reduced binding to hen egg lysozyme compared to D1.3.--

Please replace the paragraph beginning at page 94, line 12, with the following rewritten paragraph:

E18 --In vitro mutagenesis of fdT Bst was used to generate vectors having appropriate restriction sites that facilitate cloning of antibody fragments downstream of the gene III signal peptide and in frame with the gene III coding sequence. The oligonucleotide directed mutagenesis system version 2 (Amersham International) was used with oligo 1 (figure 4A) to create fdTPs/Bs (to facilitate cloning of VH fragments). The sequence of fdTPs/Bs (figure 4B, line B) was confirmed using the Sequenase version 2.0 kit (USB Corp., PO Box 22400, Cleveland, Ohio, 44122, USA.) with oligo 3 (figure 4A) as a primer.--

Please replace the paragraph beginning at page 94, line 24, with the following rewritten paragraph:

E19 --A second vector fdTPs/Xh (to facilitate cloning of single chain Fv fragments) was generated by mutagenising fdTPs/Bs with oligo 2 (figure 4A) according to the method of Venkitaraman, A.R., Nucl. Acid Res. 17, p 3314. The sequence of fdTPs/Xh (figure 4B, line C) was confirmed using the sequenase version 2.0 kit (USB Corp.) with oligo 3 (figure 4A) as a primer.--

Please replace the paragraph beginning at page 95, line 29, with the following rewritten paragraph:

E20 --The plasmid scFv D1.3 myc (gift from G. Winter and A. Griffiths) contains VH and VL sequences from the antibody D1.3 fused via a peptide linker sequence to form a single chain Fv

E20 Cont'd version of antibody D1.3. The sequence of the scFv and surrounding sequences in scFvD1.3 myc is shown in figure 5A and figure 5B.--

Please replace the paragraph beginning at page 96, line 6, with the following rewritten paragraph:

E21 --Digestion of scFv D1.3 myc with PstI and XhoI (these restriction sites are shown on figure 5A and figure 5B), excises a fragment of 693 bp which encodes the bulk of the scFv. Ligation of this fragment into fdTPs/Xh cleaved with PstI and XhoI gave rise to the construct fdTscFvD1.3 encoding the gene III signal peptide and first amino acid fused to the complete D1.3 scFv, followed by the mature gene III protein from amino acid 2.--

Please replace the paragraph beginning at page 98, line 19, with the following rewritten paragraph:

E22 --The VH fragment from D1.3 was generated from the plasmid pSW1-VHD1.3-TAG1 (Ward, E.S. et al., 1989 supra.). Digestion of this plasmid with PstI and BstEII generates the fragment shown between positions 113 and 432 in figure 5A. Cloning of this fragment into the PstI and BstEII sites of fdTPs/Bs gave rise to the construct fdTVHD1.3 which encodes a fusion protein with a complete VH domain inserted between the first and third amino acids of the mature gene III protein (amino acid two has been deleted).--

Please replace the paragraph beginning at page 105, line 15, with the following rewritten paragraph:

E23 --The starting point for this example was the clone Fab D1.3 in pUC19, a map of which is shown in figure 10D. The regions hybridising with the oligonucleotides KSJ6 and 7 below are shown underlined in figure 10A and figure 10B. The sequence encoding the VH-CH1 region (defined at the 5' and 3' edges by the oligonucleotides KSJ6 and 7 below) was PCR amplified from Fab D1.3 in pUC19 using oligonucleotides KSJ 6 and 7, which retain the Pst I site at the 5' end and introduce a Xho I site at the 3' end, to facilitate cloning into fd CAT2. The sequences for the

Sub Q19

E23 omitted

oligonucleotides KSJ6 and 7 are shown below. The underlined region of KSJ7 shows the portion hybridising with the sequence for D1.3.--

Please replace the paragraph beginning at page 109, line 15, with the following rewritten paragraph:

E24

--Enrichment was also demonstrated using purely immunological criteria. For example, 10^{12} phage (at a ratio of 1 pAb (D1.3) to 4 x 10^6 fdTPs/Bs) was subjected to two rounds of affinity selection, and then 26 colonies picked and grown overnight. The phage was then assayed for lysozyme binding by ELISA (as example 6). Five colonies yielded phage with lysozyme binding activities, see table 1, and these were shown to encode the scFv (D1.3) by PCR screening (example 13, using 30 cycles of 1 minute at 92°C, 1 minute at 60°C, 1 minute at 72°C using CDR3PCR1 and oligo 3 (figure 4A) as primers).--

Please delete the blank space on page 111.

Please replace the paragraph beginning at page 112, line 3, with the following rewritten paragraph:

E25

--Oxazolone is a hapten that is commonly used for studying the details of the immune response. The anti-oxazalone antibody, NQ11 has been described previously (E. Gherardi, R. Pannell, C. Milstein, J. Immunol. Method 126 61-68). A plasmid containing the VH and VL gene of NQ11 was converted to a scFv form by inserting the BstEII/SacI fragment of scFvD1.3 myc (nucleotides 432-499 of figure 5A) between the VH and VL genes to generate pscFvNQ11, the sequence of which is shown in fig. 13. This scFv was cloned into the Pst1/Xhol site of FdTPs/Xh (as described earlier) to generate pAb NQ11 has an internal Pst1 site and so it was necessary to do a complete digest of pscFvNQ11 with Xhol followed by a partial digest with Pst1).--

Please replace the paragraph beginning at page 118, line 7, with the following rewritten paragraph:

--DNA fragments encoding scFv's from D1.3 or NQ11 were generated by PCR using the primers shown below. These primers were designed to generate a fragment with BamH1 sites near both the terminii, to enable cloning into the BamH1 site of gene3 (see figure 16A). The oligonucleotides used, also ensure that the resulting PCR product lacks Pst1 and Xhol restriction sites normally used for manipulating the scFv's (see figure 16A). This will facilitate subsequent manipulation of a second antibody fragment in the usual way at the N terminus of gene 3. The oligonucleotides used were--

Please replace the paragraph beginning at page 120, line 1, with the following rewritten paragraph:

--It may be possible to clone into alternative sites to retain binding activity. The peptide repeats present in gene III may provide such a site (figure 16A blocks A and B). This can be done by inserting a BamHl site and using the PCR product described above. To facilitate this, the natural BamHl site was removed by mutagenesis with the oligonucleotide G3mut Bam shown below (using an in vitro mutagenesis kit (Amersham International)):--

Please replace the paragraph beginning at page 120, line 15, with the following rewritten paragraph:

--The oligonucleotide G3 Bamlink was designed to introduce a BamHl site at a number of possible sites within the peptide linker sites A and B, see figure 16B. The sequence of the linker is:--

Please replace the paragraph beginning at page 120, line 21, with the following rewritten paragraph:

--Its relationship to the peptide repeats in gene III is shown in figure 16A.--

Please replace the paragraph beginning at page 127, line 28, with the following rewritten paragraph:

-Purify on a 2% lmp (low melting point agarose/TAE (tris-acetate EDTA)gel and extract the DNA to 20 μl H_2O per original PCR using a GENECLEAN kit (see earlier; Bio101, La Jolla CA, USA) in accordance with the manufacturers instructions.--

Please replace the paragraph beginning at page 136, line 6, with the following rewritten paragraph:

--Duplicate samples of 35 μl concentrated phage were incubated with 125I-PDGF-BB (78.7fmol, 70nCi, 882Ci/mmol; Amersham International plc, Amersham, Bucks) for 1 hour at 37°C. Controls were included in which fdTPs/Bs vector phage (figure 4B, line B) or no phage replaced fd h-BDGFB-R phage. After this incubation, 10ul of sheep anti-M13 polyclonal antiserum (a gift from M. Hobart) was added and incubation continued for 30 min at 20°C. To each sample, 40ul (20ul packed volume) of protein G Sepharose Fast Flow (Pharmacia, Milton Keynes) equilibrated in PDGF binding buffer was added. Incubation was continued for 30 min at 20°C with mixing by end over end inversion on a rotating mixer. The affinity matrix was spun down in a microcentrifuge for 2 min and the supernatant removed by aspiration. Non-specifically bound ^{125I}-PDGF-BB was removed by resuspension of the pellet in 0.5ml PDGF binding buffer, mixing by rotation for 5 min, centrifugation and aspiration of the supernatant, followed by two further washes with 0.5ml 0.1% BSA, 0.2% Triton-X-100. The pellet finally obtained was resuspended in 100ul PDGF binding buffer and counted in a Packard gamma counter. For displacement studies, unlabelled PDGF-BB (Amersham International) was added to the stated concentration for the incubation of ^{125}I-PDGF-BB with phage.--

Please replace the paragraph beginning at page 147, line 27, with the following rewritten paragraph:

--To sequence clones, template DNA was prepared from the supernatants of 10 ml cultures grown for 24 hours, and sequenced using the dideoxy method and a Sequenase kit (USB), with primer LINKFOR (see example 14) for the VH genes and primer fdSEQ1 (5'-GAA TTT TCT GTA

TGA GG) for the Vk genes. Twenty-three of these hapten-binding clones were sequenced and eight different VH genes (A to H) were found in a variety of pairings with seven different Vk genes (a to g) (figure 24A through figure 24D). Most of the domains, such as VH-B and Vk-d were 'promiscuous', able to bind hapten with any of several partners.--

Please replace the paragraph beginning at page 148, line 10, with the following rewritten paragraph:

--The sequences of the V-genes were related to those seen in the secondary response to phOx, but with differences (figure 24A through figure 24D). Thus phOx hybridomas from the secondary response employ somatically mutated derivatives of three types of Vk genes - Vkoxl. 'Vkox-like' and Vk45.1 genes (C. Berek, G. M. Griffiths & C. Milstein Nature 316 412-418 (1985). These can pair with VH genes from several groups, from Vkoxl more commonly pairs with the VHoxl gene (VH group 2. R.Dildrop uupra). Vkoxl genes are always, and Vkox-like genes often, found in association with heavy chains (including VHoxl) and contain a short five residue CDR3, with the sequence motif Asp-X-Gly-X-X in which the central glycine is needed to create a cavity for phOx. In the random combinatorial library however, nearly all of the VH genes belonged to group 1, and most of the Vk genes were ox-like and associated with VH domains with a five residue CDR3, motif Asp/Asn-X-Gly-X-X (figure 24A through figure 24D). Vkoxl and VHoxl were found only once (Vk-f and VH-E), and not in combination with each other. Indeed Vk-f lacks the Trp91 involved in phOx binding and was paired with a VH (VH-C) with a six residue CDR3.--

Please replace the paragraph beginning at page 151, line 9, with the following rewritten paragraph:

--The promiscuity of the VH-B and Vk-d domains prompted the applicants to force further pairings, by assembling these genes with the entire repertoires of either Vk or VH genes from the same immunised mice. The resulting 'hierarchical' libraries, (VH-B x Vk-rep and VH-rep x Vk-d), each with $4x10^7$ members, were subjected to a round of selection and hapten-binding clones isolated (Table 4). As shown by ELISA, most were strong binders. By sequencing twenty-four clones from each library, the applicants identified fourteen new partners for VH-B and thirteen for Vk-d (figure

E 34 Contd

24A through figure 24D). Apart from VH-B and Vk-c, none of the previous partners (or indeed other clones) from the random combinatorial library was isolated again. Again the Vk genes were mainly ox-like and the VH genes mainly group 1 (as defined in Dildrop, R. 1984 supra), but the only examples of Vkoxl (Vk-h, -p, -q and -r) have Trp91, and the VH-CDR3 motif Asp-X-Gly-X-X now predominates. Thus some features of the phOx hybridomas seemed to emerge more strongly in the hierarchial library. The new partners differed from each other mainly by small alterations in the CDRs, indicating that much of the subtle diversity had remained untapped by the random combinatorial approach. More generally it has been shown that a spectrum of related antibodies can be made by keeping one of the partners fixed and varying the other, and this could prove invaluable for fine tuning of antibody affinity and specificity.--

Please replace the paragraph beginning at page 211, line 15, with the following rewritten paragraph:

E 35

--After 4 rounds of mutation and selection, isolated clones were screened and in one or two rare examples strongly positive ELISA signals were obtained from phage antibodies derived from the mutation of each of fdCAT2scFvB18 and fdDOGKanscFvB18 in the ELISA. The ELISA conditions were such that the parent phage fdCAT2scFvB18 only generated weak signals. These phage antibodies giving strongly positive ELISA signals were enriched in further rounds by a factor of roughly 2.5 per round. Forty phage antibodies giving strongly positive signals were sequenced and they each displayed single mutations in six different positions in the scFvB18 nucleotide sequences, five of which reside in the light chain. More than 70% of the mutations occurred at positions 724 and 725 changing the first glycine in the J segment of the light chain (framework 4) to serine (in 21 cases) or aspartate (in 3 cases). The mutations found are shown in Table 9. The sequence of scFvB18 is shown in figure 44A and figure 44B.--

Please replace the paragraph beginning at page 218, line 13, with the following rewritten paragraph:

E 36

--The overall strategy for the PCR assembly is shown in Figure 47 and is described in more detail below. For Fab assembly, the VH-CH1 and VK-CK or V lambda-C lambda light chains are

E36 contd amplified from first strand cDNA and gel purified. Heavy and light chain DNA are then combined together with linker DNA and flanking oligonucleotides in a new PCR reaction. This results in a full length Fab construct since the 5' end of the linker DNA is complementary to the 3' end of the CH1 domain and the 3' end of the linker is complementary to the 5' end of the light chain domain. The linker DNA contains terminal residues of the human CH1 domain, the bacterial leader sequence (pelB) for the light chain and the initial residues of the VK or V lambda light chain (figure 2A and figure 2B). Finally, after gel purification, the Fab construct is reamplified with flanking oligonucleotides containing restriction sites for cloning.--

Please replace the paragraph beginning at page 221, line 18, with the following rewritten paragraph:

E37 --To make the Fab linker DNA, 13 separate PCR reactions were performed using HulgG1-4CH1FOR and each of the reverse VK or V lambda oligonucleotides. The template was approximately 1ng of pJM-1Fab D1.3 (figure 48A) The PCR reaction reagents were as described above and the cycle was 94 :1 min, 45 :1min and 72 :1 min. The linkers were analyzed on a 4% agarose gel, purified on a 2% agarose gel, eluted from the gel on a Spin-X column and ethanol precipitated.--

Please replace the paragraph beginning at page 252, line 22, with the following rewritten paragraph:

E38 --A dilution series was made on 10 clones which were analysed by ELISA in 6 of these clones the profile of binding to HEL was the same as the original clone (pCAT3SCFvD1.3) while the signal with TEL was increased (see figure 50A clone B1). In the remaining 4 clones, the increased signal with TEL was accompanied by a decrease in signal on HEL (see figure 50A clone A4).--

In the Claims

60, 61, 62, 63, 64, 65, 102, 103, 104, 105, 106, 107, 108 and 109 and 145